Proc. Natl. Acad. Sci. USA
Vol. 77, No. 8, pp. 5580–5582, October 1980
Chemistry

Self-association and base pairing of guanosine, cytidine, adenosine, and uridine in dimethyl sulfoxide solution measured by ^{15}N nuclear magnetic resonance spectroscopy

(nucleosides)

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Contributed by John D. Roberts, May 22, 1980

ABSTRACT **The self-association of guanosine, cytidine, and adenosine and base pairing between guanosine, cytidine, adenosine, and uridine in dimethyl sulfoxide have been investigated by the variation of their ^{15}N NMR chemical shifts with concentration and temperature. Guanosine, cytidine, and adenosine all showed evidence of self-association by hydrogen bonding. In guanosine/cytidine mixtures, a hydrogen-bonded dimer is formed; however, no base pairing could be detected with adenosine/cytidine or adenosine/uridine mixtures.**

The structures of nucleic acids are featured by hydrogen bonds formed between purine and pyrimidine bases linking together polynucleotide strands. Several ^1H NMR investigations have been made of base-pair interactions of the component nucleosides of the nucleic acid chains (1–4). These indicate that guanosine and cytidine form base pairs stabilized by three hydrogen bonds, as proposed in the structure of nucleic acids by Watson and Crick (5). Base pairing between adenosine and uridine or adenosine and thymidine is expected to be stabilized by only two hydrogen bonds (5). This weaker interaction has been detected by Shoup *et al.* (1) in dimethyl sulfoxide, but is more readily observable in dimethyl sulfoxide/benzene mixtures (2) or in chloroform (2). Furthermore, ^1H NMR measurements have shown that guanosine and cytidine self-associate to form dimers in dimethyl sulfoxide (3). Self-association has also been found with adenosine, but not uridine (4).

Recently, ^{15}N NMR spectroscopy has been employed to study the general characteristics of nucleoside resonances (6, 7) and, more specifically, to investigate interactions between base pairs (7, 8). ^{15}N NMR is expected to have an advantage for the latter purpose through being especially sensitive to hydrogen bonding at azine-type nitrogens (6). The early ^{15}N NMR study of Hawkes *et al.* (7) involved only the uridine–adenosine interaction in dimethyl sulfoxide, and no effect was detected on the ^{15}N chemical shift. However, a subsequent investigation (8) of a mixture of a substituted uridine and a substituted adenosine in deuteriochloroform showed a significant downfield shift (4.73 ppm), which was ascribed to a base-pair interaction.

In the present investigation of hydrogen bonding in self-association and base-pair formation, the ^{15}N chemical shifts of nucleosides alone and in mixtures were determined in dimethyl sulfoxide as a function of concentration and temperature.

EXPERIMENTAL SECTION

The nucleosides were commercial materials and were used without further purification. The ^{15}N NMR spectra were recorded with a Bruker WH-180 spectrometer, operating at 18.25 MHz, with 1 M $^2H^{15}NO_3$ in ^2H_2O or 1 M $^1H^{15}NO_3$ in ^1H_2O used as an external reference. Hexadeuteriodimethyl sulfoxide added to the solutions provided an internal deuterium field-frequency lock. Pulse widths of 60 μsec and repetition rates of 20 sec were commonly used. The spectra were taken with proton decoupling, but with suppression of the nuclear Overhauser enhancement (6).

RESULTS AND DISCUSSION

Because dimethyl sulfoxide hydrogen-bonds well to proton donors, nucleosides are expected to be substantially associated with the solvent. However, with increasing nucleoside concentration, self-association should play an increasingly important role and cause changes in ^{15}N chemical shifts of those nitrogen nuclei involved in the self-association bonding. On addition of a complementary nucleoside, significant changes in ^{15}N shifts should occur if the base pairing for those interactions is greater than the self-association interactions. Data on the ^{15}N shifts for mixtures of guanosine and cytidine, adenosine and cytidine, and adenosine and uridine at several temperatures in the range 20–60°C are collected in Tables 1–3.

Guanosine. Two possible models (G_2 and G_2') for self-association of guanosine involving two hydrogen bonds are shown in Fig. 1. In both structures, N7, N1, and NH_2 are involved in hydrogen bonding. As the concentration of guanosine is increased from 0.4 M to 0.9 M, the only significant change in the ^{15}N chemical shifts is the shift of N7, which moves upfield by 1.9 ppm. This shift change is consistent with self-association of guanosine by hydrogen bonding to N7. The small upfield changes in ^{15}N shifts of N1 and NH_2 of 0.1 and 0.2 ppm, respectively, neither prove nor disprove involvement of N1 and NH_2, because these nitrogens are proton donors in hydrogen-bond formation and, unlike N7, can hydrogen bond to dimethyl sulfoxide as well as to other guanosine molecules. Consequently, no large changes in their ^{15}N shifts are expected as the result of switching from association with the solvent to association with another guanosine molecule. Four different dimer structures of guanosine have been proposed (3), only two of which (G_2 and G_2' of Fig. 1) directly involve N7. The fact that the chemical shift of N7 is substantially affected by concentration changes lends support to the latter structures, in agreement with the earlier conclusions drawn from ^1H NMR results by Newmark and Cantor (3).

In mixtures of guanosine and cytidine, the shift of the guanosine N7 appears to depend more on the guanosine than on the cytidine concentration. From this, it appears that N7 is involved in self-association but not in base pairing. With increasing ratio of cytidine to guanosine, complementary base pairing of guanosine is expected to increase. This is a strong three-hydrogen-bond association by the Watson and Crick formulation, and now it is not surprising to find small downfield shifts. However, neither the N1 nor the -NH_2 are types of nitrogens whose ^{15}N shifts are particularly sensitive to hydrogen bonding.

The temperature dependences of the guanosine N7 and N1 shifts are not large enough to draw any very clear conclusions.

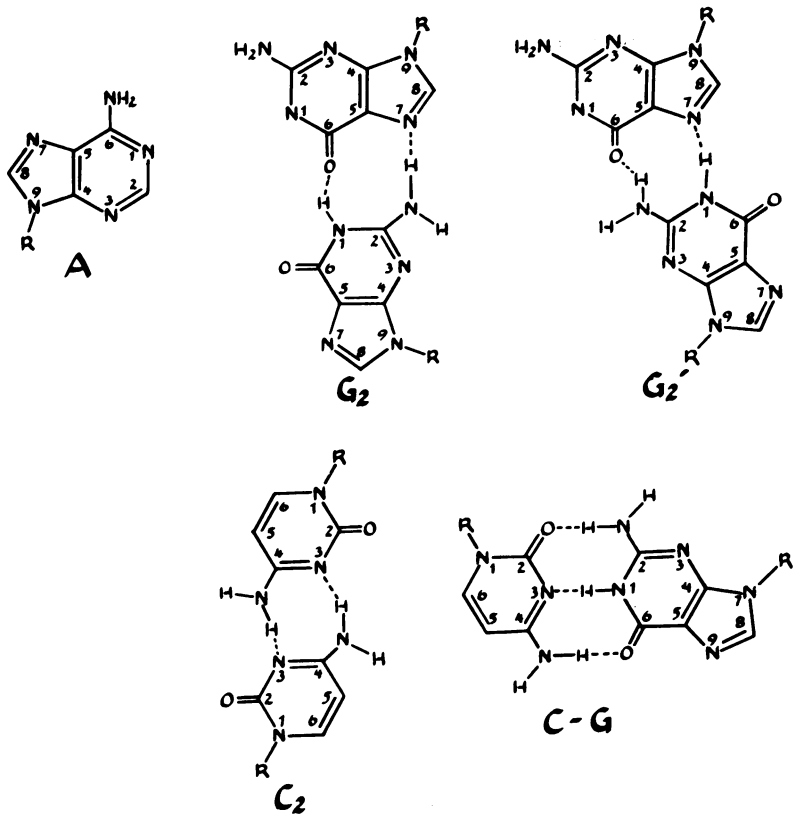

FIG. 1. The structure of adenosine (A), the proposed dimer structures of guanosine (3) (G_2 and G_2') and cytidine (C_2), and the cytidine-guanosine base-pair structure of Watson and Crick (C–G). R, ribose.

One would expect the temperature dependence of the NH_2 shift to become larger at the higher concentration of cytidine to guanosine because of the increase in base pairing, but instead it decreases.

Cytidine. As the concentration of cytidine is increased, the change in the N3 shift parallels that of N7 in guanosine. This shows that N3 is involved in self-association of cytidine, in agreement with the dimerization structure shown in Fig. 1. The shift of the -NH_2 nitrogens, which is the other nucleus expected to be affected by association, moves somewhat downfield in the same way as that of the NH_2 nitrogen when guanosine dimerizes.

Table 1. Concentration and temperature dependence of ^{15}N chemical shifts of guanosine alone and mixed with cytidine

Conc., M	N1	N3	N7	N9	NH_2
Concentration dependence					
		Chemical shift, ppm*			
0.41†	227.7	209.0	126.9	204.9	301.8
0.6†	227.8	209.0	127.7	205.0	301.8
0.9†	227.8	209.0	128.8	205.0	301.6
0.8:0.4‡	227.6	208.8	129.3	205.1	301.0
0.8:0.8‡	227.4	208.8	130.1	205.2	300.6
0.4:0.8‡	227.2	208.7	128.8	205.2	—
Temperature dependence					
		Change in chemical shift, ppm/10°C§			
0.6†	0.0	−0.1	−0.2	—	0.2
0.9†	0.0	−0.1	−0.2	0.0	0.2
0.8:0.4‡	0.0	−0.1	−0.2	0.0	0.2
0.8:0.8‡	0.0	−0.1	−0.2	0.0	0.1
0.4:0.8‡	0.0	−0.1	−0.2	0.0	0.1

* Upfield of external 1 M $^1H^{15}NO_3$ in ^1H_2O.
† Guanosine.
‡ Gyanosine:cytidine.
§ Corrected for the temperature dependence of the reference (0.08 ppm/10°C over the 20–60°C range).

Table 2. Concentration and temperature dependences of ^{15}N chemical shifts of cytidine alone, mixed with guanosine, or mixed with adenosine

Conc., M	N1	N3	NH_2
Concentration dependence			
		Chemical shift, ppm*	
0.36†	222.1	—	281.6
0.6†	222.1	164.9	281.4
0.9†	222.2	165.9	281.3
0.4:0.8‡	222.2	169.4	280.7
0.8:0.8‡	222.3	171.6	280.3
0.8:0.4‡	222.2	172.4	280.2
0.4:0.4§	222.1	165.7	281.2
Temperature dependence			
		Change in chemical shift, ppm/10°C¶	
0.6†	0.0	−0.3	0.3
0.9†	0.0	−0.4	0.3
0.4:0.8‡	0.0	−0.5	0.2
0.8:0.8‡	0.0	−0.5	0.3
0.8:0.4‡	0.0	−0.6	0.1
0.4:0.4§	0.0	−0.2	0.3

* Upfield of external 1 M $^1H^{15}NO_3$ in ^1H_2O.
† Cytidine.
‡ Guanosine:cytidine.
§ Adenosine:cytidine.
¶ Corrected for the temperature dependence of the reference; see Table 1.

Table 3. Concentration and temperature dependences of ^{15}N chemical shift of adenosine alone, mixed with cytidine, or mixed with uridine

Conc., M	N1	N3	N7	N9	NH_2
Concentration dependence					
		Chemical shift, ppm*			
0.6†	139.2	152.4	134.4	205.6	293.2
0.9†	139.8	152.7	134.9	205.7	293.1
0.4:0.4‡	139.6	152.6	134.7	205.6	293.4
0.4:0.4§	139.6	152.6	134.8	205.6	293.4
Temperature dependence					
		Change in chemical shift, ppm/10°C¶			
0.6†	−0.1	−0.3	0.0	0.0	0.4
0.9†	−0.1	−0.3	0.0	0.0	0.5
0.4:0.4‡	−0.1	−0.2	0.0	0.0	0.4
0.4:0.4§	−0.1	−0.2	0.0	0.0	0.4

* Upfield from external 1 M $^2H^{15}NO_3$ in ^2H_2O.
† Adenosine.
‡ Adenosine:cytidine.
§ Adenosine:uridine.
¶ Corrected for the temperature dependence of the reference; see Table 1.

In mixtures containing cytidine and guanosine, the cytidine N3 nucleus moves upfield by 3.5 ppm at a guanosine-to-cytidine ratio of 1:2. A further upfield shift of 3.0 ppm occurs when the ratio is increased to 2:1. The cytidine -NH_2 nitrogen shift moves somewhat downfield, whereas that of N1 remains virtually unaltered. These results are in agreement with the formation of a Watson–Crick base pair, which involves N3 and NH_2 in cytidine.

The N3 chemical shift is temperature sensitive, and the sensitivity increases at the higher concentrations of cytidine, as expected for association. For cytidine/guanosine mixtures, the temperature dependence is still larger, providing further evidence for base pairing. The temperature dependence of the NH_2 shift is erratic, in the same way observed for the NH_2 shift of guanosine, in that it does not increase uniformly as the extent of base pairing increases.

When cytidine is mixed with adenosine, there are only very small changes in the N3 andNH_2 shifts, and if any association occurs between these bases, it hardly involves N3 in an important way.

Adenosine. The chemical shifts of N1, N3, N7, and NH_2 of adenosine (Table 3) change with concentration in a way that indicates at least some self-association of adenosine. Because N1, N3, and N7 all seem involved, it is possible that several different adenosine dimers are formed. When either cytidine or uridine is mixed with adenosine, the changes in chemical shift are minimal, indicating no significant amount of base pairing. In a Watson–Crick base pair, the shifts of N1 and NH_2 should be most affected, while in the base-pair structure suggested by Hoogsteen (9), those of N7 and NH_2 would be involved. That neither of the shifts expected to be most sensitive (N1 or N7) shows significant change indicates that neither form of base pairing is very important in dimethyl sulfoxide solution. It will be seen from Table 3 that the temperature dependences of the shifts of N3 and NH_2 are large compared to those for corresponding nitrogens of guanosine and cytidine. This may mean that adenosine is largely self-associated by intermolecular hydrogen bonding between N3 and NH_2 groups.

This is contribution no. 6283 from the Gates and Crellin Laboratories of Chemistry. These studies were supported by the National Science Foundation and by the U.S. Public Health Service, Grant GM-11072 from the Division of General Medical Sciences. The National Science Foundation also provided a Summer Undergraduate Research Fellowship (Grant EPP 75-04744) for P.P.P.

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